Stability of Drugs and Dosage Forms

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Chapter 5

Stability of Peptide and Protein Pharmaceuticals

Chapters 2, 3, and 4 concerned the stability of pharmaceuticals containing pharmacologically active ingredients of relatively low molecular weight. This chapter addresses the stability of peptide and protein drugs. Peptides and proteins can undergo some of the same degradation processes seen in small molecules. However, the stability of protein and peptide pharmaceuticals can be affected by additional reactions that alter their tertiary or higher structures.

Like drugs of low molecular weight, peptides and proteins undergo chemical degradation pathways such as hydrolysis and racemization. Depending on their molecular weight, they are also susceptible to physical degradation by denaturation, aggregation, and precipitation. Because of the complicated degradation mechanisms, it is generally more difficult to predict the stability of peptide and protein pharmaceuticals.

Chemical and physical properties of peptides and proteins have been studied extensively. The thermodynamics of protein structure have also been studied in detail and reported in many excellent reviews and books.[786–788] The present chapter focuses on the stability of peptide and protein pharmaceuticals upon storage.

5.1. Degradation of Peptide and Protein Pharmaceuticals

Degradation observed with peptide/protein pharmaceuticals is classified into chemical and physical mechanisms. The former involve changes in covalent bonds, and the latter involve changes in noncovalent interactions such as hydrophobic bonding/associations. For a specific peptide/protein, degradation usually includes both chemical and physical pathways as well as interactive pathways that might result when a molecule undergoes intermolecular disulfide exchange accompanied by precipitation. This section outlines each of the major chemical and physical degradation pathways.

5.1.1. Chemical Degradation

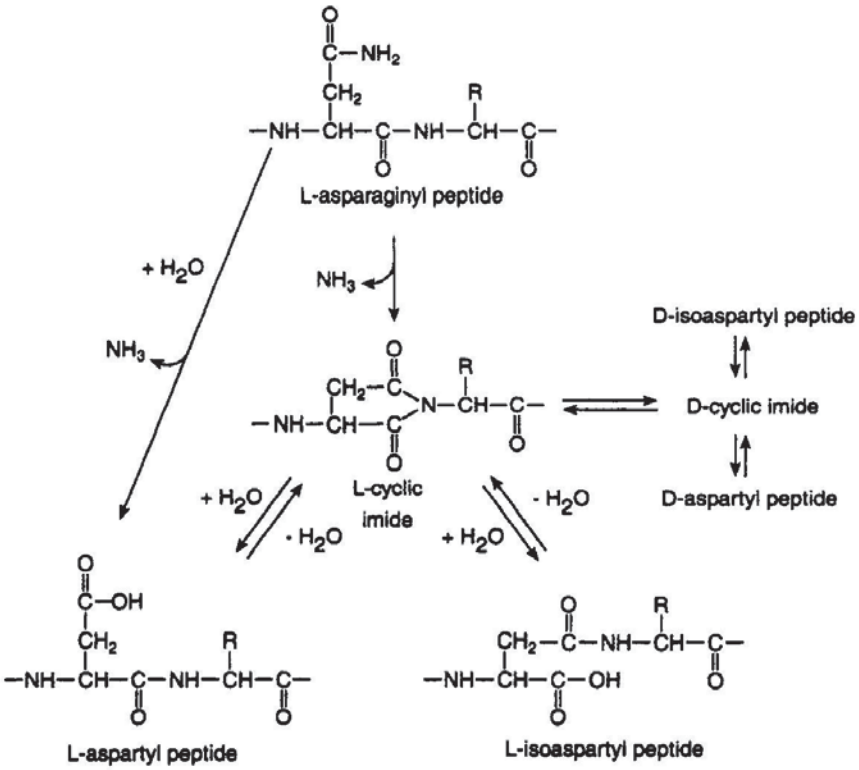
The major known chemical degradation pathways for peptides and proteins are deamidation, racemization, isomerization, hydrolysis, disulfide formation/exchange, β-elimination, and oxidation.

5.1.1.1. Deamidation

Asparagine residues in peptides and proteins undergo deamidation via cyclic imide formation followed by subsequent hydrolysis to form the corresponding aspartic and *iso*-aspartic acid peptides. This mechanism occurs primarily under neutral-to-basic pH conditions. Deamidation of an asparagine residue to the corresponding aspartic acid residue may also occur via a mechanism that does not involve cyclic imide formation, as shown in Scheme 80. Glutamine residues also undergo deamidation, but at much slower rates.

Adrenocorticotropic hormone (ACTH), which has 38 amino acid residues, exhibited pseudo-first-order deamidation in the neutral-to-alkaline pH region. The deamidation rate increased with increasing pH and buffer concentration. Deamidation via the cyclic imide of an asparagine residue was suggested since both the aspartic acid and *iso*-aspartic acid peptides were detected as deamidation products. As shown in Table 13, the rate of disappearance of ACTH showed good mass balance with the rates of appearance of deamidated ACTH and ammonia, indicating that the rate-determining step for the deamidation is not degradation of the cyclic imide but its formation.[789] A model hexapeptide with an asparagine residue (Asn-hexapeptide) exhibited a similar deamidation reaction. The deamidation rate was higher for asparagine residues having a smaller amino acid at the C-terminal side of the residue, as shown in Table 14, indicating that steric factors may influence cyclic imide formation.[790,791]

Deamidation of ACTH under acidic pH conditions is considered to be direct deamidation to the aspartic acid peptide since the *iso*-aspartic acid peptide was not observed as a



Scheme 80. Scheme showing the deamidation, isomerization, and racemization of peptides having asparagine or aspartic acid residues.

Table 13. The Effect of Glycine Buffer Concentration on the Deamidation of ACTH at pH 9.6 and 37°C[a]

	Apparent rate constant (h^{-1}) at pH 9.6, 37°C and glycine buffer concentration of:		
	10m*M*	50m*M*	100m*M*
Disappearance of ACTH	6.6×10^{-2}	1.4×10^{-1}	2.3×10^{-1}
Appearance of deamidated ACTH	4.7×10^{-2}	1.4×10^{-1}	2.6×10^{-1}
Appearance of ammonia	6.5×10^{-2}	1.6×10^{-1}	2.9×10^{-1}

[a] Reference 789.

degradation product.[789] Similar direct deamidation of the model Asn-hexapeptide resulted in 100% formation of the aspartic acid peptide.[790,791]

Insulin has two asparagine residues that undergo deamidation. At acidic pH values, Asn A-21 undergoes deamidation, whereas at neutral pH and in suspensions, deamidation at residue Asn B-3 predominates.[792] Deamidation of insulin at pH 2 and 3 was also enhanced by self-association.[793]

5.1.1.2. Isomerization and Racemization

Peptides and proteins having an aspartic acid residue undergo hydrolysis, isomerization, and racemization via cyclic imide formation. As shown in Scheme 80, L-aspartic acid peptide can isomerize to L-*iso*-aspartic acid peptide via its L-cyclic imide. The L-cyclic imide intermediate is capable of undergoing racemization to the D-cyclic imide and thus forms the D-aspartic acid peptide and the D-*iso*-aspartic acid peptide on hydrolysis.

Following storage of a secretin solution, aspartoyl[3] secretin (cyclic imide) and β-aspartyl[3] secretin (isomer) were detected in the solution by reversed-phase HPLC, indicating that isomerization occurred via the cyclic imide.[794,795] An Asp-hexapeptide also exhibited isomerization via cyclic imide formation at pH values above 4.[796] The rate of formation of the cyclic imide was affected by the size of the amino acid on the C-terminal side of the aspartic acid residue.[797] A cyclic imide was also detected as a major degradation product of basic fibroblast growth factor at pH 5.[798]

Table 14. The Effect of the Amino Acid Residue on the C-terminal Side of Asn on the Deamidation of Asn-Hexapeptides[a]

Asn-hexapeptide	t_{50} (days)
Val-Tyr-Pro- Asn-Gly- Ala	1.89 (pH 7.5)
Val-Tyr-Pro- Asn-Ser- Ala	5.55 (pH 7.5)
Val-Tyr-Pro- Asn- Ala- Ala	20.2 (pH 7.4)
Val-Tyr-Pro-Asn-Val- Ala	106 (pH 7.5)
Val-Tyr-Pro- Asn-Pro-Ala	70 (pH 7.4)
Val-Tyr-Pro- Asn-Pro- Ala	106 (pH 7.4)

[a] Reference 791.

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